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Supplementary Text

For the years up to and including 2012, the multilocus genotype (MLG) of each *P. ramorum* strain was determined based on microsatellite analysis of five loci, PrMS6, Pr9C3, PrMS39, PrMS45 and PrMS43, using previously published protocols (Grünwald et al. 2009; Prospero et al. 2004, 2007; Grünwald et al. 2008). Multilocus genotyping of *P. ramorum* strains collected in 2013 and 2014 included an extra nine loci, KI18, KI64, KI82a, KI82b, ILVOPrMS79, ILVOPrMS131, ILVOPrMS145a, ILVOPrMS145b, ILVOPrMS145c which are amplified by an additional six primer pairs (Ivors et al. 2006; Vercauteren et al. 2011, 2010). The locus ILVOPrMS79, amplifies up to three alleles, however two separate loci have yet to be described (Vercauteren et al. 2011). The addition of nine loci to the genotyping assay coincided with the discovery by the Oregon Department of Agriculture of an EU1 *P. ramorum* isolate in a Curry County nursery in 2012. Preceding 2012, only NA1 isolates had been found in Curry County. Because different loci are polymorphic for different clonal lineages, the entire panel of 14 loci was necessary to adequately describe the *P. ramorum* population in the event that multiple lineages were discovered in the forest.

Methods for genotyping the 2013 and 2014 *P. ramorum* strains with all 14 loci use new multiplex protocol. Previously published primers were modified by the addition of a 5′ PIG tail "GTTT" to reverse primers in an effort to reduced stutter peaks and hence to better facilitate allele scoring (Table 2)(Brownstein et al. 1996). In two cases (PrMS45, PrMS6), a PIG tail was not added to reverse primers to simplify scoring of overlapping alleles. Also,

where a T residue was already present at the 5′ end of a reverse primer, as in the case of KI18, a "GTT" was added instead of "GTTT". Forward primers were assigned fluorescent labels, 6-FAM, NED, VIC, or PET, to facilitate separation of overlapping markers (Table 2). Primer concentrations were determined by visual inspection of electropherograms (Table 2).

Amplification of all 14 loci was separated into three reactions (8-plex, 2-plex and simplex). The simplex reaction amplified the PrMS43 locus using methods described earlier (Prospero et al. 2007; Grünwald et al. 2009). The 8-plex and 2-plex amplified the remaining loci and were performed under identical conditions with the exception of primers and primer concentrations (Table 2). For the multiplex reactions, the QIAGEN Type-it Mutation Detect PCR Kit (QIAGEN, 206343, Valencia, CA) was used. Multiplex PCR reactions were performed in 5µl volumes with 10ng template DNA and1X final buffer concentration. Amplifications were run on a Veriti thermal cycler (Life Technologies, Grand Island, NY) with an initial denaturation at 95 °C for 5 min, followed by 33 cycles of 95 °C for 30 s, 60 °C for 90 s, and 72 °C for 20 s, and a final extension at 60 °C for 30 min. Genotyping prior to 2012 included three reference DNA lineages (EU1, NA1, and NA2). After 2012, a fourth lineage (EU2) was added as a reference (Van Poucke et al. 2012).

Electrophoresis and visualization of all microsatellites were performed on ABI3100, ABI3100 Avant, or ABI3130 genetic analyzers (Applied Biosystems). For evaluation of the loci, genotyped prior to 2013, the PCR products were diluted 10 times in ultrapure H_20 and 1.5 µl of diluted product was added to both 8.5 µl of Hi-Di™ Formamide (Applied Biosystems, 4311320) and 0.25 µl of GeneScan™ 500 LIZ™ size standard (Applied Biosystems, 4322682). The simplex reaction (PrMS43) was also diluted 10 times while the

8-plex and 2-plex products were diluted 75 times. After dilution, 2.5 µl of the 8-plex, 2-plex and simplex products were added to 7.5 µl of Hi-Di™ Formamide containing GeneScan™ 500 LIZ™size standard at a ratio of 6 µl size standard to 1 ml Hi-Di™ Formamide. Allele sizing was determined using GeneMapper® v3.7 and v5.0 software (Applied Biosystems).

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